JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2010, p. 4598–4601 Vol. 48, No. 12
0095-1137/10/$12.00 doi:10.1128/JCM.00004-10

Evaluation of Molecular Assays for Rapid Detection of Methicillin-Resistant *Staphylococcus aureus*[▽]†

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Received 2 January 2010/Returned for modification 5 May 2010/Accepted 29 September 2010

The diagnostic sensitivities of the BD GeneOhm and Cepheid Xpert assays were compared using culture on log-serial dilutions of well-characterized methicillin-resistant *Staphylococcus aureus* (MRSA) and non-MRSA strains and on nasal and groin swabs from patients with histories of MRSA carriage. The sensitivities of GeneOhm and Xpert were high at 10^3-CFU/ml MRSA concentrations (92.3% and 96.3%, respectively) although decreased considerably (<35%) at a 1-log-lower concentration. Unexpectedly, both assays also detected select coagulase-negative staphylococci, which requires further evaluation.

Effective and rapid laboratory diagnosis is critical for treating, managing, and preventing methicillin-resistant *Staphylococcus aureus* (MRSA) infections. PCR-based MRSA detection assays offer certain benefits over conventional culture techniques, such as lower limits of detection (LoDs), high-throughput screening, and, importantly, shorter time to detection. Currently, two of the most promising commercially available PCR-based assays for MRSA detection are the GeneOhm MRSA (BD Diagnostics, Erembodegem, Belgium) and Xpert MRSA (Cepheid, Bouwel, Belgium) assays (reviewed in reference 10). Both target the junction of the mobile element staphylococcal cassette chromosome *mec* (SCC*mec*) carrying the *mecA* methicillin resistance gene in *S. aureus* (6).

(Part of this work was presented at the 19th ECCMID, 16 to 19 May 2009, Helsinki, Finland.)

We first evaluated and compared the diagnostic sensitivities of the BD GeneOhm and Cepheid Xpert MRSA assays for patient screening samples compared to culture—both direct and subsequent to overnight enrichment—on conventional/chromogenic media (mannitol salt agar with 4 μg/ml cefoxitin and BBL-CHROMagar [BD Diagnostics]), followed by confirmatory testing, as previously described (9, 21). Fifty-two nose and groin samples were prospectively collected in 1.5 ml brain heart infusion broth and 15% glycerol from 26 previously identified MRSA carriers at the University of Geneva Hospitals. Patient samples were tested according to manufacturers' recommendations by the GeneOhm and Xpert assays, showing similar sensitivities for MRSA detection (96% and 93%, respectively) compared to direct culture, which detected 28 samples as MRSA positive (Table 1). Consistent with recent reports (1, 21), an overnight enrichment protocol drastically increased the MRSA true-positive status of the patient screening samples compared to direct culture (42/52 versus 28/52 samples). For the 14 samples that did not show any MRSA CFU on direct culture, Xpert successfully detected MRSA in 2 samples and GeneOhm in 7 samples, suggesting an increased sensitivity of these PCR-based assays over direct cultures. However, when preenriched-culture results were taken as the gold standard, GeneOhm and Xpert showed significantly reduced sensitivities of 81% (McNemar test; $P = 0.039$) and 66.7% ($P = 0.001$), respectively (Table 1). However, the sensitivities of GeneOhm and Xpert were not significantly different from each other, with an overall concordance of 80.8% ($n = 42$, Cohen's kappa = 0.60), or concordances of 76.9% ($n = 20$; kappa = 0.54) and 84.6% ($n = 22$; kappa = 0.65) for nasal and groin samples, respectively. These data for previously identified MRSA carriers are similar to those for recent hospital-based studies showing comparable high sensitivities for GeneOhm and Xpert for patient screening samples from the nose/groin or throat compared to the results for direct culture but a reduced performance compared to the results for enriched culture (7, 24). Only three samples with MRSA loads of 100 CFU/ml or more were not detected by these assays. These samples included two groin samples for Xpert and a nasal sample for GeneOhm from a patient that carried MRSA only in the nose. Because certain SCC*mec* IV variants are reported not to be detected by these assays, possibly due to an altered SCC*mec* element, we performed SCC*mec* genotyping as described previously (5). SCC*mec* I was the predominant clone identified in all but two strains that harbored one each of SCC*mec* II and IV. Interestingly, the nasal sample that GeneOhm failed to identify carried SCC*mec* IV MRSA.

To identify the actual LoDs of GeneOhm and Xpert for

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▽ Published ahead of print on 13 October 2010.

TABLE 1. Sensitivities of GeneOhm and Xpert for detection of MRSA from patient screening samples in comparison to results for direct and preenriched cultures

Assay	Sample source(s)	Direct culture		Preenriched culture	
		Sensitivity (%) (95% CI)	Proportion of true-positive samples	Sensitivity (%) (95% CI)	Proportion of true-positive samples
GeneOhm	Nasal	90.90 (62.5–98.4)	10/11	71.40 (50.0–86.2)	15/21
	Groin	100 (81.6–100)	17/17	90.50 (71.1–97.4)	19/21
	All	96.40 (82.3–99.4)	27/28	81.00 (66.7–90.0)	34/42
Xpert	Nasal	100 (74.1–100)	11/11	57.10 (36.6–75.5)	12/21
	Groin	88.20 (65.7–96.7)	15/17	76.10 (54.9–89.4)	16/21
	All	92.90 (77.4–98.0)	26/28	66.70 (51.6–79.0)	28/42

divergent MRSA clones as well as to overcome the inherently low level of epidemiological diversity observed among clinical samples collected from a single hospital, we analyzed 27 distinct MRSA strains at defined concentrations. These strains harbored distinct SCC*mec* subtypes and comprised some of the most prevalent, well-characterized clonal lineages that have disseminated worldwide in hospitals and communities, including animal-associated MRSA strains that are carried by and cause disease in humans (2, 22) (see Table S1 in the supplemental material). MRSA strains were tested in these assays with serial dilutions from 10^0 through 10^5 CFU/ml (1, 10, 10^2, 10^3, 10^4, and 10^5 CFU/ml) until a positive result was obtained. Both assays showed high sensitivities for detection of pure MRSA strains at concentrations of 10^3 CFU/ml, with the average LoDs for GeneOhm (430 CFU/swab, or 4,300 CFU/ml) and Xpert (250 CFU/swab, or 3,300 CFU/ml) corroborating previous data (16, 17) (GeneOhm MRSA package insert) (Table 2). Nonetheless, the steep drop in sensitivity at 10^2 CFU/ml brings into question the ability of these assays to accurately detect MRSA carriage at lower concentrations in carriers, including carriers who have completed decolonization treatment but in whom complete eradication has not been achieved (14, 23). Moreover, 3 MRSA strains could not be detected at 10^3 CFU/ml but could be detected at a 1-log-higher concentration in two independent experiments. These strains included MRSA strains harboring SCC*mec* III/sequence type 239 (ST239) (GeneOhm; human MRSA strain 9) (Table S1), SCC*mec* IV/ST398 (GeneOhm; animal MRSA strain 19), or SCC*mec* V/ST398 (Xpert; animal MRSA strain 20). The reduced sensitivities of detection observed for these MRSA strains corroborate previous reports of detection failures for human and animal MRSA harboring SCC*mec* types III, IV, and V in these assays (8, 15, 19, 20). While the precise reason for this is unknown, sequence variations in the targeted *orfX*-SCC*mec* junction region, which are especially common in animal MRSA (13), are the most likely reason for the poor performance of the molecular assays with specific MRSA strains. Hence, from a clinical-use perspective, iterative modifications of the molecular assays based on epidemiological changes will be necessary for optimal sensitivities to be sustained.

Lastly, we also studied cross-reactions to non-MRSA strains for mixtures of select MRSA and non-MRSA strains, including various methicillin-resistant and -sensitive coagulase-negative staphylococci (MRCoNS and MSCoNS, respectively) ($n = 25$) (see Table S1 in the supplemental material, strains 28 through 52). Twenty-one mixtures of non-MRSA/MRSA strains were prepared as described in the supplemental material and assayed with serial dilutions from 10^0- to 10^5-CFU/ml MRSA concentrations. Interestingly, increased sensitivity (and decreased LoD) was observed for MRSA strains in mixtures spiked with non-MRSA strains compared to the level for pure MRSA strains at similar concentrations (Table 2). To study whether this increased sensitivity was due to cross-reactivity to non-MRSA strains, we tested all 25 pure non-MRSA strains individually as well as 8 mixtures comprising only non-MRSA strains at a single high concentration of 10^5 to 10^6 CFU/ml MRSA. Those showing false-positive results for either molecular assay were confirmed with log-serial dilutions. False-positive detections of pure non-MRSA strains and their mixtures were observed sporadically for GeneOhm (all 5 MRCoNS and 1 of 3 MSCoNS strains tested) and Xpert (3 MRCoNS and 2 MSCoNS strains) (see Table S2 in the supplemental material for the threshold cycle [C_T] values obtained for these strain dilutions). In a previous analytical study by Huletsky and colleagues, approximately 250 MRCoNS and MSCoNS strains did not show any false-positive detection with an in-house real-time PCR targeting *orfX*-SCC*mec* junction (6). GeneOhm and Xpert are also based on the same principle, although the primer targets might differ from those used by Huletsky et al. (6). A U.S.-based study tested 44 strains of MRCoNS and MSCoNS on Xpert and did not find any cross-reactivity (24), although the species and SCC*mec* types present in these strains were not described in the study. In yet another analytical study, Francois and colleagues showed false-positive results for GeneOhm with MSSA, but MRCoNS were not tested (4). Some other clinical studies with large numbers of human screening samples have also shown false-positive results; however, the underlying cross-reactive organisms could not be completely elucidated (3, 7). Interestingly, in similarity to *S. aureus*, the vicinity of the *orfX* gene is also a preferred site for insertion of SCC*mec* cassettes in other staphylococci, and frequent exchange of parts or of entire SCC*mec* elements or even of non-*mecA*-containing SCC elements is also common in these organisms (11, 12). Preliminary sequencing of the *orfX*-SCC*mec* junction region in select falsely positive MRCoNS has shown high homology to MRSA (S. Malhotra-Kumar, M. Gazin, L. Van Heirstraeten, and H. Goossens, unpublished results). Thus, in addition to the well-described cross-reactivity with MSSA (18), our study shows that the presence of select MRCoNS in human screening samples could also affect the specificity of *orfX*-SCC*mec*-targeting assays.

We thank BD Diagnostics and Cepheid for providing test kits and recommendations for patient sample and strain testing. We thank the MOSAR WP2 partners (Waleria Hryniewicz, Jordi Vila, Marek Gniadkowski, and Claire Poyart) for providing strains. S.H. and A.L. gratefully acknowledge the staff of the Staphylococcal Laboratory and the Infection Control Program at the University of Geneva Hospitals for assistance with specimen collection and processing.

This work, L.V.H., and A.L. were supported by funding from the European Community (MOSAR network contract LSHP-CT-2007-037941 and TheraEdge network contract FP7-216027). S.M.-K. is funded by the Research Foundation—Flanders (FWO-V), Belgium. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. S.M.-K. has received a speaker's honorarium from BD Diagnostics. We declare no conflict of interest.

TABLE 2. Sensitivities and limits of detection for the two assays tested on pure strains and their defined mixtures at various concentrations

Sample group (no. of samples)	Assay	LoD range (CFU/ml)	Avg LoD (95% CI)		Sensitivity (%) at indicated MRSA concn (no. of positive samples)		
			CFU/ml	CFU/swab[a]	10^2 CFU/ml	10^3 CFU/ml	10^4 CFU/ml
MRSA strains (27)	GeneOhm	1.4×10^2–4.1×10^4	4.3×10^3 (1.7×10^2–3.2×10^4)	4.3×10^2 (1.7×10^1–3.2×10^3)	33.30 (9)	92.30 (25)	100 (27)
	Xpert	1.4×10^2–2.0×10^4	3.3×10^3 (1.6×10^2–1.1×10^4)	2.5×10^2 (1.2×10^1–8.4×10^2)	14.80 (4)	96.30 (26)	100 (27)
MRSA/non-MRSA mixtures (21)	GeneOhm	5.4×10^0–5.1×10^3	2.0×10^3 (4.5×10^1–4.9×10^3)	2.0×10^2 (4.5×10^0–4.9×10^2)	42.90 (9)	100 (21)	ND[b]
	Xpert	2.7×10^1–5.1×10^3	2.4×10^3 (3.7×10^1–5.0×10^3)	1.8×10^2 (2.7×10^0–3.7×10^2)	38.10 (8)	100 (21)	ND

[a] Calculated for 100-μl and 75-μl sample inputs for GeneOhm and Xpert, respectively.
[b] ND, not determined, as all MRSA-positive mixtures were detectable at the preceding lower concentration.

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